Hepatitis B antigen: distribution of ad and ay subtypes in blood donors and hepatitis patients

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Summary: Hepatitis B antigen (HBAg) from blood donors and patients with hepatitis was tested for *ad* and *ay* subspecificity by immunodiffusion in agarose. A total of 59 sera from blood donors and 81 sera from hepatitis patients were subtyped.

Subtyping of HBAg from blood donors showed *ad* and *ay* subspecificity in 64.4 and 35.6% of cases respectively. Patients' sera on the other hand showed HBAg with *ad* and *ay* subspecificity in 6 and 94% of cases respectively. Therefore, *ad* subtype was more frequently associated with blood donors whereas *ay* subtype was predominant among hepatitis patients. The relationship between clinical findings and HBAg subtype is also discussed.

Résumé: *Antigène B de l'hépatite: répartition des sous-types* ad *et* ay *chez les donneurs de sang et les malades atteints d'hépatite*

Nous avons soumis à l'épreuve d'immunodiffusion sur agarose l'antigène B de l'hépatite (AgBH), des sérums provenant de donneurs de sang et de malades souffrant d'hépatite, en vue de déterminer les sous-groupes spécifiques *ad* et *ay*. Au total, 59 sérums provenaient de donneurs de sang et 81 de malades souffrant d'hépatite.

Les spécimens provenant de donneurs de sang ont indiqué que 64.4% étaient du sous-groupe *ad* et 35.6% du sous-groupe *ay*. Par contre, les sérums provenant de malades souffrant d'hépatite ont révélé la présence des sous-groupes *ad* et *ay* de l'AgBH dans 6 et 94% des cas respectivement. Il ressort donc de ces résultats que le sous-type *ad* se retrouve plus fréquemment chez les donneurs de sang alors que le sous-type *ay* prédomine dans les cas d'hépatite. Nous avons également étudié les rapports existant entre les constatations cliniques et le sous-type spécifique d'AgBH découvert.

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The existence of subspecificities of hepatitis B antigen (HBAg) was first demonstrated by Levene and Blumberg[1] who, on the basis of spur formation in gel diffusion, detected three determinants, a, b and c of HBAg. This was further confirmed by Kim and Tilles[2] and Le Bouvier,[3] the latter reporting the presence of a common antigenic component *a* and two mutually exclusive determinants *d* and *y*. The HBAg associated with hepatitis was of either *ad* or *ay* subtype.

Other subspecificities of HBAg have also been described such as the *w* and *r* determinants reported by Bancroft, Mundon and Russell[4] and an *e* determinant reported by Magnius and Espmark.[5] Further investigation is needed to determine the character and significance of these new determinants.

The distribution of *ad* and *ay* subtypes of HBAg varies with geographic region and clinical form of hepatitis. Schmidt, Roberto and Lennette[6] found antigens with *ad* subspecificity predominant in asymptomatic Tongans, while *ay* subtype represented almost all the antigens from various groups in California including heroin users and patients with hepatitis in a San Francisco hospital. Gordon, Berberian and Stevenson[7] found in Los Angeles that subtype *ad* predominated among hepatitis patients. Holland *et al*[8] observed a predominance (86 to 87%) of *ad* subtype in blood donors and patients with chronic hepatitis. Magnius and Espmark[5] also observed a predominance (93%) of *ad* subtype in blood donors while *ay* subtype was predominant (87%) in hepatitis patients. Variations among different groups of patients were also reported by Schober, Thomssen and Kaboth[9] who found that subtype *ad* was predominant in carriers as well as acute hepatitis B patients in northwest Germany. On the other hand an outbreak among patients and staff in the Göttingen dialysis centre showed only subtype *ay* HBAg.

Dodd *et al*[10] found regional variations among blood donors. Recently Iwarson *et al*[11] reported that *ad* subtype of HBAg was more common (56%) among blood donors while *ay* subtype was predominant (75%) in post-transfusion hepatitis patients, and considered these differences as reflecting epidemiological circumstances.

In this report additional observations on the occurrence of *ad* and *ay* subtypes of HBAg in blood donors and patients with acute hepatitis are presented.

Materials and methods

Bloor donors

Fifty-nine HBAg-positive sera from blood donors were obtained from the Canadian Red Cross Blood Transfusion Service, Toronto, and tested for *ad* and *ay* subspecificity.

Hepatitis patients

Sera were obtained from patients with hepatitis in the Ottawa Civic Hospital, Ottawa General Hospital and Montfort Hospital in Ottawa. Sera were tested in our laboratory for presence of HBAg by counterimmunoelectrophoresis (CIEP). A total of 81 sera accumulated between February 1972 and February 1973 were subtyped for *ad* and *ay* determinants by immunodiffusion in agarose. Clinical data were obtained from patients' hospital records.

Reference HBAg and antisera

Reference antigens and antisera of *ad* and *ay* subspecificity were kindly supplied by Dr. G. L. Le Bouvier of the Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut and Dr. J. W. Mosley, John Wesley Hospital, Los Angeles, California. Purified HBAg with *ad* and *ay* subspecificity was purchased from Electro-Nucleonics Laboratories Inc., Bethesda, Maryland. Anti-*ay* goat serum was kindly supplied by Dr. H. E. Bond (Electro-Nucleonics Labs). Reference *ad* and *ay* antisera were obtained from the Research Resources Branch, N.I.A.I.D., National Institutes of Health, Bethesda, Maryland.

Preparation of antisera

Anti-*ad* and anti-*ay* sera were prepared by inoculation of guinea pigs with *ad* and *ay* HBAg. Antigen was diluted 1:3 in saline and emulsified with an equal volume of complete Freund adjuvant. The first injection (0.05 ml.) was given in each foot pad, followed by four subcutaneous injections (0.2 ml.) at weekly intervals. The guinea pigs were test-bled and the serum checked for specific precipitation before final bleeding. The guinea pig anti-*ad* and anti-*ay* sera were tested against *ad* and *ay* subtype HBAg by immunodiffusion in agarose. Both sera reacted specifically with the homologous subtype and gave reactions of partial identity when tested against the heterologous subtype. The sera were also compared with the reference antisera. Testing against normal serum did not produce any precipitin line.

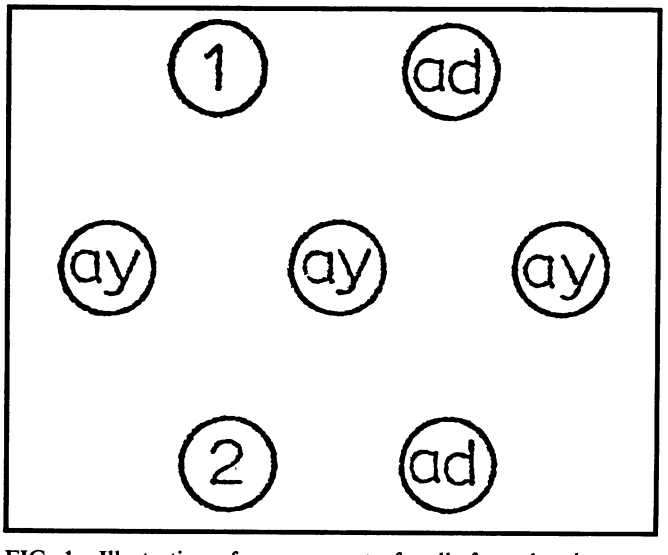

FIG. 1—Illustration of arrangement of wells for subtyping of HBAg by immunodiffusion. Guinea pig and anti-*ay* serum is placed in wells marked "ay", reference antigen in wells marked "ad", and the two sera of unknown subtype in wells marked "1" and "2" respectively.

Immunodiffusion tests

The test procedures were similar to those reported by Le Bouvier.[3] Coated microscope slides were layered with 1.8 ml. of 0.5% agarose (L'Industrie Biologique Française, Genevilliers) in 0.1 M NaCl buffered with 0.01 M Tris (hydroxymethyl) aminomethane at pH 7.6 and containing 0.1% sodium azide. Slides were incubated in a moist chamber at 22°C. for 48 to 72 hours and read unstained. Two types of reaction were observed in immunodiffusion tests, one of identity in which precipitin lines joined completely, the other of partial identity in which part of the lines joined but formed spurs.

HBAg subtyping

Subtyping was done by immunodiffusion in agarose. In the test, wells marked *ay* (Fig. 1) were filled with anti-*ay* serum, wells marked *ad* were filled with reference *ad* subtype HBAg and wells marked 1 and 2 were filled with HBAg-containing sera to be subtyped. Sera tested were considered of *ay* subtype if a spur was formed in contact with the adjacent *ad* subtype reference antigen. If sera produced a line of identity, they were considered as *a* or *ad* subtype. Sera were tested simultaneously against *ad* antiserum by replacing the anti-*ay* serum in the centre wells with anti-*ad* serum and the *ad* antigen with *ay* reference antigen. In this case spur formation in contact with adjacent *ay* antigen identified the serum as *ad* while a reaction of identity would have identified the serum tested as subtype *a*.

Table I — Distribution of HBAg subtypes *ad* and *ay* in blood donors and hepatitis patients

Category	No. of cases	HBAg subtype	
		ad	*ay*
Blood donors	59	38 (64.4%)	21 (35.6%)
Hepatitis patients	81	5 (6.0%)	76 (94.0%)

Table II — Distribution of HBAg subtypes *ad* and *ay* in different clinical forms of hepatitis

Clinical form of hepatitis	No. of patients	HBAg subtype	
		ad	*ay*
Acute	54	2 (4%)	52 (96%)
Chronic active	5	1 (20%)	4 (80%)
Post-transfusion	3	—	3 (100%)
Hemodialysis	4	2 (50%)	2 (50%)
Unknown*	15	—	15 (100%)

*Specimens received from hospital without sufficient data.

Table III — Frequency of HBAg subtypes *ad* and *ay* in relation to drug abuse

Category	Clinical form of hepatitis	No. of patients	HBAg subype	
			ad	*ay*
Drug abusers	Acute	37	—	37(100%)
	Chronic active	4	—	4(100%)
Those denying drug abuse	Acute	17	2(12%)	15(88%)
	Chronic active	1	1(100%)	—

Sera producing a weak precipitin line and which could therefore not be subtyped were concentrated (5- to 12-fold) by ultracentrifugation.

Results

HBAg subtypes in blood donors and patients with hepatitis

The results of HBAg subtyping of sera from 59 blood donors and 81 hepatitis B patients are given in Table I. The HBAg-positive sera were of either *ad* or *ay* subtype; none of the sera tested showed single antigenic determinants. The subtypes were determined by spur formation.

The distribution of *ad* and *ay* subtypes of HBAg was different in blood donors and hepatitis patients. The donors showed a higher percentage of *ad* subtype (64.4%) while in patients with hepatitis *ay* subtype was predominant (94%).

Distribution of HBAg subtypes in relation to clinical forms of hepatitis

Subtyping of HBAg from patients with hepatitis (Table II) showed a predominance of *ay* subtype in cases of acute hepatitis (96%) and chronic active hepatitis (80%). Clinical records were not available for 15 of the patients, most of whom were outpatients and could not be followed up. In all these patients *ay* subtype of HBAg was found. All patients with post-transfusion hepatitis showed also *ay* subtype.

Among the four patients on hemodialysis, two with subclinical hepatitis had an *ad* subtype. Of the remaining two, one had clinical hepatitis with jaundice and the other periarteritis; both had *ay* subtype HBAg.

Distribution of HBAg subtypes in relation to drug abuse

Differences in the distribution of HBAg subtypes were observed between patients abusing parenteral drugs and those denying drug abuse (Table III). All 41 drug abusers with either acute or chronic active hepatitis had an *ay* subtype HBAg. Among patients with acute hepatitis denying drug abuse, 88% had an *ay* subtype and 12% an *ad* subtype. The only patient with chronic active hepatitis who denied drug abuse had an *ad* subtype. She had a history of hepatitis seven years before her present illness.

Discussion

The present studies indicate that subtype *ay* of HBAg is prevalent in patients with hepatitis while *ad* subtype was more often associated with asymptomatic carriers (blood donors). Similar findings have been reported earlier by Schmidt, Roberto and Lennette,[6] Holland *et al*,[8] Magnius and Espmark[5] and Iwarson *et al*.[11] On the other hand, Gordon, Berberian and Stevenson[7] observed a predominance of *ad* subtype among patients with hepatitis. Dodd *et al*[10] also found the *ad* subtype predominating in blood donors although they could observe regional variations in the *ad*/*ay* ratio.

The analysis of the distribution of HBAg subtypes in different forms of hepatitis confirms the predominance of *ay* subtype among patients with clinical hepatitis. The group of "unknown" patients represents most probably cases of acute hepatitis. The distribution of HBAg subtypes in this group is also strikingly similar to that in the group of patients with acute hepatitis.

It might be of interest to note that the two hemodialysis patients with *ad* subtype had a subclinical infection while the two with *ay* subtype had some clinical manifestation, *viz.* jaundice and periarteritis.

All 41 patients with hepatitis known as drug abusers had an *ay* HBAg. Of the 17 patients with acute hepatitis who denied parenteral abuse of drugs nine gave a history of contact with cases of acute hepatitis within a few months prior to their illness and one was a hospital laboratory technician handling blood samples. Fifteen of these patients had an *ay* subtype of HBAg.

It appears that subtyping of HBAg can play an important role in the epidemiological studies of hepatitis type B infections. Kim and Tilles[2] have described a number of cases in which the same antigenic subtype could be found in cases of hepatitis as in their contacts. Le Bouvier[12] has pointed out that all recipients of serum pool MS-2 at Willowbrook State School had an *ay* subtype of HBAg.

Our data suggest that epidemiological factors play an important role in the relative frequency of HBAg subtypes in hepatitis patients and blood donors. Further studies are necessary to clarify the role of HBAg subtypes in determining the clinical characteristics of the infection.

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